A STUDY OF CHEMORECEPTION IN AQUEOUS AND GAS PHASES[1]

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Although it is generally recognized that chemoreception plays an important part in the orientation, feeding and reproductive activities of many aquatic animals (Jahn and Wulff, 1950; Walker and Hasler, 1949), these animals have been a perplexing group in developing concepts of the physiology of the chemical senses. At first it was even questioned whether presumed chemical sense organs of aquatic organisms could have any function at all, since they were surrounded by a fluid medium rather than a gaseous one (Nagel, 1894). Nagel's negative conclusion, based upon anthropomorphic considerations, has long been discredited by experiments indicating that many reactions of aquatic animals are mediated by receptors which he held to be functionless—*e.g.,* the receptors in the olfactory pits and connected to the olfactory nerves (Parker and Sheldon, 1913; von Frisch, 1924).

Matthes (1924), interested in adaptations of the sensory apparatus to different environments, then raised the question whether the same receptors could mediate reactions to chemical stimuli underwater and on land in the case of the animals which spent a portion of their life cycles in each environment. It was found that *Triton* no longer reacted to chemicals in either environment when the olfactory nerve was cut. The olfactory innervation was traced to the Jacobson's organ (Matthes, 1927) and it was shown that the presumed receptors in the larval and adult animals were histologically very similar to those of other vertebrates. When it was discovered that a sheet of mucus, variable in quantity and distribution according to the physiological state of the animal, overlay the olfactory receptors (Murphy, 1931; Leasure, 1939) the logical assumption that chemical stimuli went into solution in the mucus layer abridged the usefulness of the previous observations on vertebrates for understanding what specializations, if any, of the receptors existed for functioning in gas and liquid phases. Schaller (1926) avoided this difficulty by studying amphibious beetles which have chemoreceptors on mouthparts accessible for ablation experiments, and which could be tested both underwater and with a dry cuticular surface in air. Conditioned reactions of *Dytiscus* to coumarin and synthetic musk, either underwater or in air, ceased after removal of the flagellar portion of the antennae and the maxillary palpi. Acid, salt, and sugar were similarly detected by receptors on the labial palpi and the inside of the mouth. It was concluded that different groups of receptors were specialized for perception of either of two types of chemical stimuli—those which are "odor-substances" for man (irrespective of physical state of the stimulus), or those compounds which are

[1] The author takes pleasure in acknowledging his indebtedness to Dr. V. G. Dethier of the Johns Hopkins University for the loan of some of the equipment used in this work, and for his critical reading of the manuscript.

"taste-substances" for man, with secondary specializations for perceiving particular modalities of taste substances, such as acid, salt, sugar, and bitter compounds, since these compounds are discriminated by *Dytiscus*. However, conflicting conclusions based upon experiments with the same and related species of insects were presented by Ritter (1936) and Bauer (1938). Although accepting Schaller's main concept about the types of physiological specializations to be found in chemoreceptors, they did not agree on the location or morphology of the receptor groups sensitive to "odor-substances," "taste-substances," or the various modalities of the latter group. Ritter (1936) reported that the antennae of *Hydrous* completely lacked chemoreceptors and that heliotropin and skatol stimulated receptors on the tips of the maxillary palpi; the receptors of the labial palpi were reported to be sensitive only to acid. Bauer (1938) failed to prevent reaction to non-acidic stimuli by removing the maxillary palpi. Nor was agreement reached on the morphological appearance of the variously located and specialized receptors.

In a recent review, Dethier and Chadwick (1948) discussed possible sources of conflicting data in the experiments cited above (such as the lack of quantitative control of the stimulus) and have concluded that the relationship between the physical state of a chemical and its effect on chemoreceptors is unknown. They further conclude that this relationship should be explored not only to understand what specializations, if any, differentiate receptors functioning in gas and liquid phases and the sensitivity to various modalities of stimuli in each phase, but also to determine whether the same limiting mechanism operates in both phases. The aim of the present work is to determine, first of all, whether the same set of receptors mediates reactions to chemical stimuli administered in gas and liquid phases and whether different groups of receptors are specialized for perception of particular modalities of stimuli in either phase. The question of the fundamental limiting mechanism in each phase is later discussed.

Materials and Methods

The experimental animal was an amphibious beetle, *Laccophilus maculosus* Germar, chosen because of its dual air-water habitat, accessible mouthparts, and availability. It has been shown (Hodgson, 1951) that *Laccophilus* possesses much lower reaction thresholds to a wide variety of chemical stimuli than those reported for other insects. Since the relations between molecular structure, chemical properties, and relative stimulating effectiveness of compounds included in several series of organic and inorganic chemicals determined for *Laccophilus* are essentially identical with the findings obtained in experiments with *Phormia* (Dethier and Chadwick, 1948), *Balanus* (Cole and Allison, 1930) and other terrestrial and aquatic invertebrates, it was concluded that at least the limiting mechanisms of chemoreception in *Laccophilus* were not aberrant and might yield information of general significance. The beetles were collected at the Fish Hatcheries Experiment Station of the United States Fish and Wildlife Service at Leetown, West Virginia. They were obtained in large numbers both in shallow water of the fish rearing ponds and in dry grass near the ponds. The methods used for maintaining stocks of these animals have been previously described (Hodgson, 1951). Every precaution was taken to standardize feeding and handling of the beetles and after being once used in the experiments the beetles were discarded.

The technique for determining threshold concentrations of pure chemicals administered in the gas phase or in the liquid phase is essentially the same, although a different apparatus is required for each of the two types of test. The over-all procedure is to apply moving, unmixed streams of air or water, containing known concentrations of the chemical to be tested, to groups of beetles and determine the percentage of beetles which react to the chemical by moving into the control area of the apparatus which is exposed to a moving stream of air or water identical in all respects except that the test compound is absent. Technical details of the construction and operation of the apparatus for determining threshold concentrations of chemicals in aqueous solution have been fully presented (Hodgson, 1951) and hence will not be repeated, since the method used here was identical. The apparatus for determining threshold concentrations of gases was a slight modification of the olfactometer described in detail by Dethier and Yost (1952), and used by these investigators in experiments with the blowfly, *Phormia*. In brief summary of this method, animals to be tested are placed in a cage through which pass two streams of air, one containing a known concentration of the gaseous chemical being tested. Before the experiment, the animals are distributed randomly in the two halves of the cage exposed to the experimental and control streams of air. Those reacting to the gas being tested move to the control side of the cage. The percentage doing so is determined by photographs taken during the run. With *Laccophilus*, it was found that the best results were obtained using a smaller cage (4″ × 2″ × 3/8″) than was used with *Phormia*. This narrow cage was placed horizontally in the apparatus so that the air stream passed through it from top to bottom, since *Laccophilus* did not climb the vertical walls of the cage as readily as did *Phormia*. Fifty unanesthetized beetles, completely dry on the external surface of the cuticle, were placed in the cage at one time. Since the number of beetles reacting is equal to the difference between the average number of beetles remaining on the experimental side of the cage and the average number originally on that side (about 25 beetles), the formula used to obtain the percentage reaction to any particular concentration was as follows: $\%\ \text{reaction} = \frac{25 - x}{25}$, where x is the number of beetles remaining on the exposed side of the cage. Aside from the slight modification of the test cage construction, this procedure was identical with that described by Dethier and Yost (1952), and their report may be consulted for technical details of the apparatus, sample calibration curves, etc.

With either aqueous or gas phase tests, doubling concentrations of the chemical stimulus are administered. The percentage of beetles reacting at each concentration is converted into probit units by the method of Bliss (1938). This conversion merely transforms the typical sigmoid dosage-reaction curve to a straight line, in which the 50% reaction point corresponds to a probit of 5. The direct relationship between the logarithm of the molar concentration of the stimulant and the percentage of beetles reacting is shown in Figure 1. The data used in this plot were selected simply because the thresholds in these two particular cases fell within a range convenient to include on a single graph. From such a plot, the 50% reaction point (threshold) can be read directly. The standard error of the threshold is obtained by the method of Miller and Tainter (1944). The tabulated results presented below are derived from data on 69 individual beetles tested in three different runs in

the liquid phase, and from data on 150 individuals tested in three different runs in the case of the gas thresholds. This difference in numbers results from the different sized populations convenient to test in the different sized reaction chambers of the two types of apparatus. An analysis of the variance between reactions of the three different groups of beetles tested at each concentration of stimulus showed no significant difference between the reactions of the three groups in either phase and the data were therefore combined and treated as though derived from a single homogeneous population.

When beetles were to be operated upon, they were anesthetized with carbon dioxide and lifted onto clay blocks, having depressions into which the animals fit with their ventral sides uppermost. A broad rubber band around the block covered the posterior halves of the beetles and held them in position during the operations.

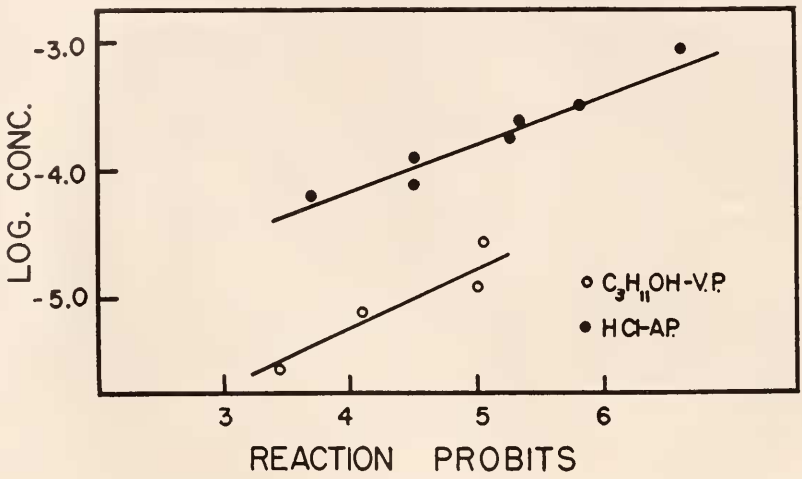

FIGURE 1. Relationship between percentage reaction and quantity of stimulating chemicals. Data in this plot obtained from experiments on unoperated beetles exposed to 1-pentanol in the vapor phase and HCl in the aqueous phase.

Watchmaker's forceps and iridectomy scissors were used to grasp and remove whatever portions of the head appendages were desired. The point at which the appendages were severed when they were to be completely removed is indicated in Figure 2. Other operations were performed as noted blow. The effects of the anesthetic wore off within five minutes and the operated beetles seemed to have no difficulty swimming or flying.

To restrict the investigation to a manageable size, the chemicals used were restricted to 1-pentanol, HCl, and NaCl. The first two compounds can be conveniently worked with in the vapor phase and enough data on their thermodynamic and other properties exist to permit comparisons of possible mechanisms of action in the two phases. The latter two compounds represent classic modalities (acid and salt) of taste stimuli which were the source of the conflicting data cited in the introduction. It should be noted that although it would have been desirable to include a sugar among the aqueous phase stimulating compounds, *Laccophilus* does

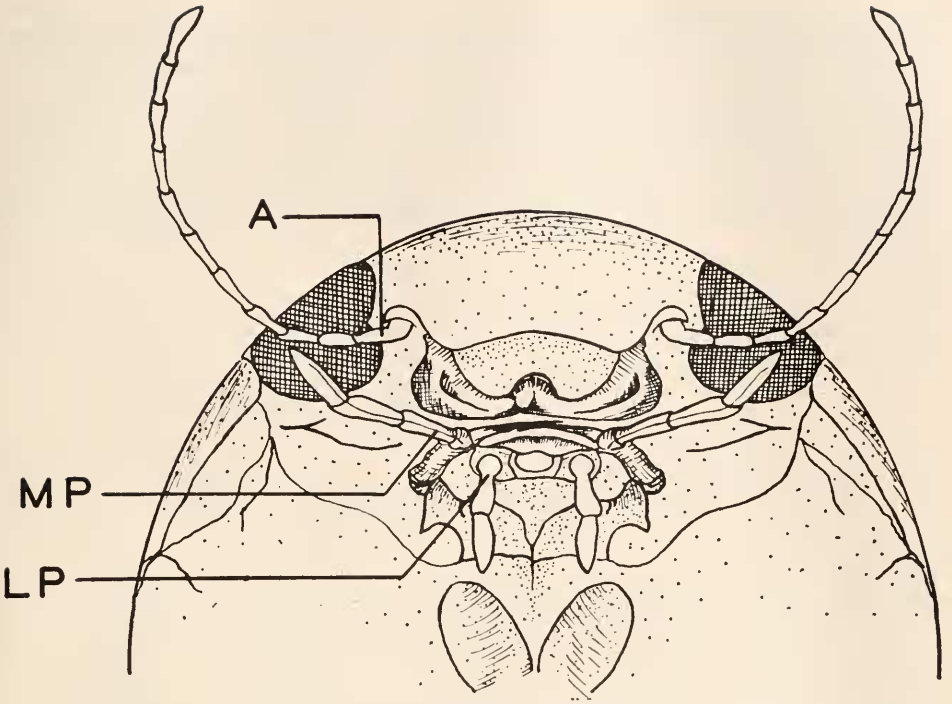

FIGURE 2. Ventral view of *Laccophilus* head, showing antennae and palpi. The pointers indicate the positions near the base of each appendage where they were severed if they were to be entirely removed.

not react to aqueous solutions of sucrose when tested by this method with the highest concentration it is possible to use in the apparatus and still maintain normal rates of flow (approximately one molar sucrose).

RESULTS

1. *The identity of receptors mediating reactions in the gas and liquid phases.* (Threshold data are summarized and compared in Tables I and II.)

Beetles were exposed to 1-pentanol and HCl in both phases and thresholds determined for the normal (unoperated) animals. As noted above, data from three groups are treated as one homogeneous unit to derive each threshold value. The results are given in Table I, Columns 1A and 1B. Then the antennae were completely removed from another series of animals and a similar run made in both phases (Table I, Columns 8A and 8B). Since it appeared that a complete loss of sensitivity to the gaseous stimuli, within the range of concentrations it was possible to test with the present apparatus, was suffered by beetles with antennae removed, it was not necessary to go on to other mouthparts in attempting to localize the receptors mediating reactions to gases, and a more precise localization of the gas phase receptors on the antennae was sought.

After several preliminary experiments, it was found that the smallest area which it was possible to cut accurately from the distal ends of the antennae was one-half of the distal segment. When the extreme distal half segments of the antennae were removed from 150 beetles, the animals gave no reaction to either 0.0003 molar pentanol, or to 0.0002 molar HCl, administered as gases. These were the highest concentrations of these compounds which it was possible to administer with the olfactometer. Therefore, it was concluded that the chemoreceptors mediating reactions to gaseous stimuli are located on the distal halves of the distal segments of the antennae.

TABLE I

Aqueous and vapor phase thresholds of Laccophilus

	Threshold (molar concentration evoking reaction from 50% of beetles) ± standard error. Appendages listed at top of columns indicate parts removed.								
	1. Control (unoperated)	2. MP LP	3. MP LP ½S−A	4. A LP	5. A LP ½S−MP	6. A MP	7. A MP ½S−LP	8. A	9. A MP LP
A. Aqueous phase									
1-pentanol	0.0019± 0.0003	0.0015± 0.0007	*	0.0082± 0.0022	*	0.0076± 0.0010	*	0.0050± 0.0007	*
HCl	0.0014± 0.0006	0.0019± 0.0010	*	0.012± 0.005	*	0.0090± 0.0008	*	0.0073± 0.0006	*
NaCl	0.17± 0.03	0.18± 0.08	*	0.78± 0.13	*	0.73± 0.19	*	0.48± 0.09	*
B. Vapor phase									
1-pentanol	0.00015± 0.00008	0.00027± 0.00011	—	—	—	—	—	*	—
HCl	0.00012± 0.00003	0.00014± 0.00005	—	—	—	—	—	*	—

Abbreviations and symbols used: A, antennae; MP, maxillary palpi; LP, labial palpi; S, distal segments; *, no reaction to highest concentration tested.

To see if the same area was involved in mediating reactions to these chemicals in aqueous solution, the distal halves of the distal segments of the antennae were removed from 69 other beetles, previously deprived of palpi—which, it will be shown, bear other chemoreceptors—and these beetles exposed to pentanol and HCl (Table I, Column 3A). These beetles no longer reacted, even to stimuli as concentrated as 0.1 molar pentanol and 0.5 molar HCl, which are, respectively, fifty and three hundred times as concentrated as would be necessary to stimulate 50% of the unoperated animals.

At first it was not known where the other receptors mediating reactions to chemicals in the liquid phase were located. On the basis of Schaller's experiments it was expected that they would be located on the palpi and in the mouth cavity.

Accordingly, the antennae and labial palpi were removed from a group of beetles, leaving only the maxillary palpi. The thresholds of this group were significantly higher than for the normal animals (Table I, Column 4A; Table II, Column 3). Removal of the distal half of the distal segment of the maxillary palpi in another group of beetles, previously deprived of antennae and labial palpi, abolished reactions to chemical stimuli at the highest concentrations mentioned above (Table I, Column 5A). Hence, it was concluded that only the distal half-segments of the maxillary palpi bear chemoreceptors. Further operative subdivision of this segment was not technically possible.

Next, the antennae and maxillary palpi were completely removed from a group of beetles, leaving the labial palpi. The threshold again increased significantly as compared with the normal animals (Table I, Column 6A; Table II, Column 4), but

TABLE II

Comparison of thresholds of receptor groups in Laccophilus

Compound	Receptor areas or operative conditions being compared. The upper figure of each set is the difference in molar threshold, and the lower figure the standard error of the difference							
	1. Normal; minus A	2. Normal; A	3. Normal; MP	4. Normal; LP	5. MP; MP+LP	6. LP; MP+LP	7. LP; MP	8. Normal; A (gas phase)
1-pentanol	0.0031 0.00095 *	0.0005 0.0011	0.0063 0.0022 *	0.0057 0.00105 *	0.0032 0.0023	0.0026 0.0013	0.0006 0.0024	0.00012 0.0080
HCl	0.0059 0.00085 *	0.0005 0.0012	0.0096 0.005 *	0.0076 0.0010 *	0.0057 0.0050	0.0017 0.0010	0.003 0.0051	0.00002 0.000044
NaCl	0.31 0.095 *	0.01 0.085	0.61 0.13 *	0.56 0.19 *	0.30 0.16	0.25 0.21	0.05 0.23	— —

An asterisk indicates a significant difference (more than twice the standard error of the difference) between thresholds. Abbreviations as in Table I. Based on tests in aqueous phase except as otherwise noted.

did not differ significantly from the threshold of the receptors on the maxillary palpi alone (Table II, Column 7), or from the threshold of the combined maxillary and labial palpi (Table II, Column 6). Removal of the distal segments of the labial palpi from a group of beetles previously deprived of their antennae and maxillary palpi abolished all reaction to the highest concentrations of chemical stimuli tested (Table I, Column 7A). In the case of the labial palpi, too, the chemoreceptors must be on the distal segment, although the small size of this segment precluded any further localization by ablation techniques.

The comparisons presented in Table II show that the only significant changes in threshold occur when the antennae of the beetles are removed, regardless of the phase in which the stimulus is administered. The high concentrations to which the "refractory" beetles were exposed without obtaining any reaction make it extremely improbable that the beetles ever employ chemoreceptors within the buccal cavity or elsewhere on the body in these experiments.

2. The specificity of the chemoreceptor groups

From the data already presented, it appears unlikely that any one appendage bears chemoreceptors sensitive to only one particular modality of stimulus, as suggested by Ritter (1936). As additional evidence on this point, the aqueous phase thresholds of the antennae and palpi to NaCl were determined and the results are included at appropriate places in Table I. It is clear that the receptor area most sensitive to HCl and pentanol (distal tip of the antennae) is also the most sensitive to NaCl, and that the palpi are again of approximately equal sensitivity, as was the case with the alcohol and acid. When the tips of the antennae were removed from animals previously deprived of palpi, the beetles gave no reaction to 1.5 molar NaCl, the highest concentration tested. The question of specialization of various receptors within a single group or area is considered in the discussion.

3. Structure of the chemoreceptors

On the basis of evidence thus far presented, it is clear that the chemoreceptors must be located within the distal halves of the distal segments of the antennae, maxillary palpi, and labial palpi. The greater sensitivity of the antennae in both phases suggests that some difference might exist either in the morphological characteristics of the receptors on the antennae and palpi or that the number of receptors might be greater on the antennae. A morphological study of the antennae and palpi was made to discover if any such specializations of structure or number of receptors were apparent. Some of the appendages removed from the operated animals were used for this purpose, and some parts were removed especially for this purpose so that their orientation on the slides could be checked.

The antennae and palpi were placed in a drop of Clarite on a microscope slide and the slide placed in a vacuum chamber. A fifteen-pound vacuum was sustained in the chamber for twenty minutes. This removed any air bubbles formed within the cut end of the material to be observed. After cover slips were in place, the whole-mounts were observed with magnifications up to 1300 ×. Camera lucida drawings of the distal segment, or portions thereof, from the antennae and palpi are shown in Figure 3. All the parts are drawn as they would be seen from the ventral side when the orientation corresponds to that shown in Figure 2. Since, in the living beetles, these appendages are usually waving around vigorously, this manner of orientation may be clearer than trying to orient their surfaces with reference only to the dorsal and ventral aspects of the beetle's body. The magnification scale for *A* and *B* is given in the upper left of the figure and the scale for *C* in the upper right. The entire distal segments of the palpi are shown, and the remainder of the distal antennal segment lacked other receptors.

The key to the presumed sense organs, following the classification of Snodgrass (1935), is as follows: 1-sensilla chaetica; 2-sensilla basiconica; 3-sensilla coeloconica; 4-sensilla placoidea. The sensilla basiconica of the palpi are the same in appearance as those on the antennae, although it was necessary to represent them diagrammatically on this drawing because of their numbers, which are at least several hundred on each palpus. Snodgrass describes the sensilla basiconica as probable chemoreceptors, but little experimental evidence on this point is available. Perhaps the most pertinent evidence is that of Roth and Willis (1951) which strongly indicates that the sensilla basiconica are hygroreceptors in *Tribolium*. It will be

noted that the arrangement of receptors in *Laccophilus* makes it practically certain that the sensilla basiconica are the chemoreceptors with which the present experiments are concerned. The only other morphologically distinguishable receptors found in all three of the receptor-bearing areas are the sensilla chaetica and other evidence, as noted below, makes their importance in chemoreception very highly improbable. For example, the sensilla chaetica were found on the other flagellar segments of the antennae and were morphologically and numerically identical on the other segments to those on the distal segment, as well as occupying similar positions on the segments. The same is true for the coeloconic sensillum which is also

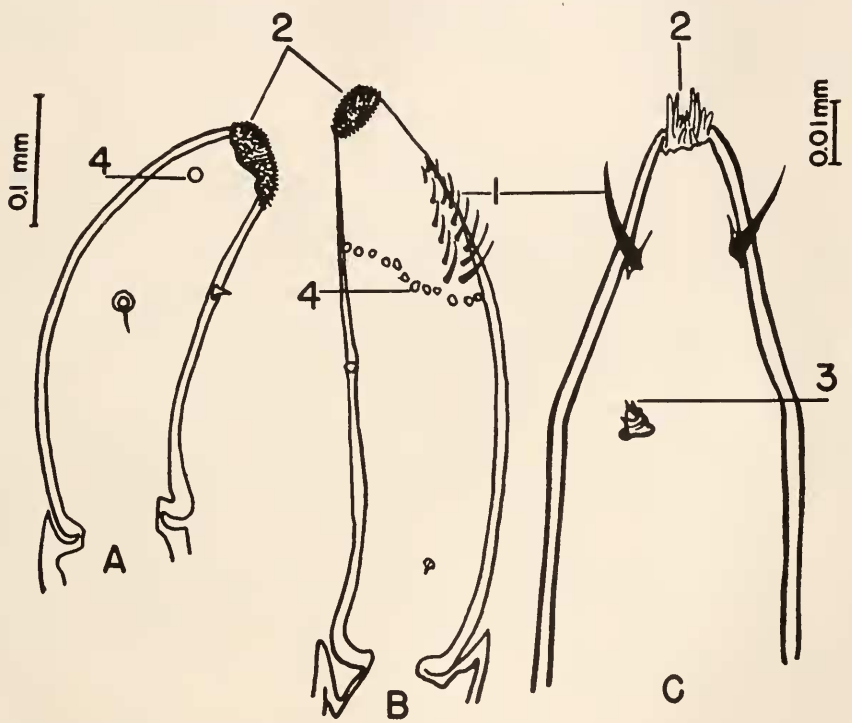

FIGURE 3. Receptors on antennae and palpi. A—distal segment of labial palp; B—distal segment of maxillary palp; C—a portion of the distal segment of an antenna. Key to receptor types and additional explanation in text.

present on other flagellar segments of the antennae. Hence, if receptors other than the sensilla basiconica were chemoreceptors on the antennae, it would be necessary to assume that similar morphological types of receptors situated in corresponding positions on other antennal receptors were functioning in some entirely different manner. This hypothesis is rejected in favor of the simpler alternative—*i.e.*, that the sensilla basiconica of the antennae are chemoreceptors, and that the other receptor types which are also present elsewhere are not chemoreceptors. The evidence with regard to the palpi is more equivocal. Sensilla chaetica are more abundant (by a factor of four) on the proximal segment of the labial palpi than on the distal segment, and are at least as abundant on the two basal segments of the maxillary

palpi as on the distal segment. Hence, it is unlikely that they are chemoreceptors. The sensilla placoidea are also possible chemoreceptors on the palpi. However, since only one of these organs is present on the distal segment of the labial palpi, it seems unlikely that it alone would be responsible for all the sensitivity of the labial palpi to chemicals. In the case of the maxillary palpi, where the placoid organs circle the distal segment, the placoid organs may be chemoreceptors.

It may be concluded that the sensilla basiconica are the chemoreceptors on the antennae and the receptors chiefly responsible for the sensitivity of this species to gaseous and liquid stimuli. There is also evidence that sensilla basiconica are chemoreceptors on the palpi. It is surprising that the most sensitive region contains the smallest number of sensilla basiconica (tips of antennae) compared to other regions where these receptors are found. The significance of this will be considered in the discussion.

Discussion

Three points merit amplification before drawing final conclusions from this study. The first of these is an assumption which underlies the experiments. This is the assumption that measurements based upon reactions of an entire animal may be used to understand the sensitivity of the animal's receptors. The possibilities for error in making this assumption are probably more obvious than the evidence of its soundness. In the first place, we might expect the assumption to be valid in view of the generalization from comparative psychology that the correlation between structure of the nervous system and behavior of animals becomes clearer as one considers experimental material along phyletic lines from mammals down through the invertebrates (Schneirla, 1952). More pertinent in this particular case is the group of observations already existing on insect chemoreception which shows excellent correlations between known physical and chemical properties of the stimulating compounds and the thresholds for reaction to them (Dethier and Chadwick, 1948). If unknown factors in the nervous systems of the experimental animals were entering into the picture, one would expect that no such clear correlations would be obtained, for they would be obscured by factors within the nervous system modifying the consistent relation between properties of the stimulus applied and the animal's reaction to them.

There is no doubt that electrical measurement of nerve impulses from the chemoreceptors would be the method of choice for use on invertebrates as well as vertebrates, but thus far this has been either technically impossible (Jahn and Wulff, 1950) or limited to detection of narcotic effects which are not specific effects of the stimulating chemicals on chemoreceptors (Hodgson and Roys, unpublished). It is hoped that eventually it will also be technically possible to limit chemoreception studies to a single receptor cell, but in the meantime much of interest may come to light while working toward that end.

In view of the finding that a very small group of antennal receptors mediates reactions to different modalities of chemicals in either gas or liquid phases, it seems logical to next inquire why the receptors on the palpi were not observed to mediate reactions in both phases, since they were sensitive to all three test compounds in the aqueous phase. This discrepancy is resolved by the observation that the thresholds of the palpi are significantly higher than those of the antennae and are, in fact, higher than the concentrations of chemicals which it is possible to accurately ad-

minister with the present olfactometer, which reaches its upper limit of concentrations at about one doubling concentration higher than the threshold of these beetles to either pentanol or HCl vapors. Although it is theoretically quite conceivable that some reactions might be obtained by exposing the receptors on the palpi to very concentrated gases, it seems quite certain that in nature the high thresholds of the palpi would prohibit their participation in mediating reactions of the animals to gaseous stimuli.

The difference in threshold obtained when the antennal receptors were tested in gas and liquid phases is, however, of some interest with regard to possible limiting mechanisms in each phase. If the same factor limits the effectiveness of one chemical in stimulating chemoreceptors in two phases, then it should be possible to bring the differences in thresholds into agreement by correcting for the difference in that factor in the two phases. Since some of the information now available on chemoreception suggests that the process is characterized by the establishment of an equilibrium between the concentration of stimulant at the basic site of action and the concentration of stimulant in the phase external to the organism (Dethier and Yost, 1952), it was thought that the limiting factor determining the thresholds in the two phases might be establishment of such an equilibrium. If this were true, the thermodynamic activity of the stimulant, suitably defined, should have the same numerical value in both phases (Ferguson and Pirie, 1948). On the basis of the present experiments, it is possible to make four such comparisons between thermodynamic activities of stimuli at threshold concentrations in aqueous and vapor phases. These values were calculated for pentanol and HCl at threshold concentrations for unoperated beetles, and for beetles with the palpi removed, according to the methods of Ferguson and Pirie (1948), and Brink and Posternak (1948). Since the thermodynamic activities at threshold were not even of the same order of magnitude when data from similarly treated beetles in the two phases were compared, this approach will not be elaborated upon here.

The essential point is that either chemoreception does not depend upon the establishment of an equilibrium in the two phases or else some behavioral difference in the testing methods used in the two phases masks the identity of the fundamental mechanisms. In this connection it is interesting to note that Dethier and Yost (1952) found that alcohols of intermediate chain length when stimulating blowflies as gases obeyed the law of equal effect at equal thermodynamic activity; however, this principle did not hold when the tarsal receptors of the blowfly are stimulated by alcohols, although here, too, the behavioral criteria might be involved in the discrepancy.

Finally, the bearing that these findings have on the concepts of olfaction, gustation, and sensory modalities should be mentioned. It must be conceded that classifying stimuli as odor-substances or taste-substances is simply making a difficult problem more obscure and is a practice which should be abandoned. In view of the present results, the advisability of making a distinction between olfactory and gustatory receptors on the basis of the physical state of the stimulus to which they are sensitive could be similarly misleading, since a very restricted group of receptors of the same morphological type can mediate reactions to both gaseous and liquid stimuli. It is remarkable that the smallest number of sensilla basiconica is on the region most sensitive to chemicals and a much larger number on the palpi which are relatively insensitive. This indicates that the sensitivity of the antennal

receptors is the result of some specialization of the receptor cells themselves rather than simply the result of a great many receptors summing nerve impulses as is sometimes advanced by way of an explanation for the greater sensitivity of olfaction as compared with gustation (Moncrieff, 1944).

There is no evidence in these data for the existence of topographically separated receptors sensitive only to particular modalities of stimuli as was concluded by Ritter (1936). It is possible that the apparent specificity of the receptors on the labial palpi for acids resulted from having the concentrations of salt and non-acid stimuli below threshold, while the acid happened to be above the threshold of the receptors on the labial palpi. This particular point is more relevant to understanding chemoreception in insects than to concepts about the chemical senses in general, since it merely indicates that the sense organs of insects have not evolved the same types of specializations as those found in mammals. Of course it cannot be assumed that different sensilla within a particular group necessarily have identical physiological functions and thresholds, any more than it could be assumed, for example, that different sensilla with cuticle of similar thickness have the same permeability properties (Richards, 1952). Such a condition would be, however, quite different from the grouping of specialized receptors suggested by Ritter (1936). This leaves the problem of the existence of modalities among chemicals stimulating for insects, and especially the basis of their discrimination by the animal, in a very unsatisfactory state. If different classes of chemicals do not activate different receptors, how are they discriminated as classes of stimuli? One wonders how many such classes of compounds might be found in a systematic experimental survey not organized around the classic modalities of taste stimuli for humans. Much additional evidence on this point is needed, but the suggestion by Frings (1946) that the modalities represent only points in a continuous spectrum of taste sensations related to some surface-active property of the chemicals might offer a less anthropomorphic approach for experimentation than some of those used in the past.

Summary

1. Quantitatively controlled stimuli were administered to populations of an amphibious beetle, *Laccophilus maculosus* Germar, to determine whether the same chemoreceptors are sensitive to gaseous and liquid stimuli, and to discover qualitative or quantitative specializations in the function of different receptor groups.

2. Sensilla basiconica on the tips of the antennae are the principal chemoreceptors for both gaseous and liquid stimuli. The lower threshold of antennal receptors, relative to receptors elsewhere on the animals, is not due to a larger number of sensilla on the antennae and indicates an inherent specialization of the receptors themselves.

3. Morphological and experimental evidence strongly indicates that the sensilla basiconica on the tips of the maxillary and labial palpi also function as chemoreceptors for stimuli in solution, although their thresholds are higher than those of antennal receptors. The concentration of gases could not be raised to a level adequate for stimulation of receptors on the palpi and they must play little or no part in mediating reactions of the animals in air.

4. HCl, 1-pentanol, and NaCl all stimulated receptor areas on the tips of the antennae and palpi, and no evidence was found for specialization of any morphologically or topographically distinguishable receptor groups sensitive to only a particular

modality of stimulus. Chemoreceptors were not found on parts of the body other than the antennae and palpi.

5. The findings are discussed with reference to possible identity of fundamental mechanisms limiting the effectiveness of chemical stimuli in the two physical states, and general concepts of olfaction and gustation.

LITERATURE CITED

Bauer, L., 1938. Geschmäcksphysiologische Untersuchungen an Wasserkäfern. *Zeitschr. vergl. Physiol.*, **26**: 107–120.

Bliss, C. I., 1938. The determination of the dosage-mortality curve from small numbers. *Quart. J. Pharm. and Pharmacol.*, **11**: 192–216.

Brink, F., and J. M. Posternak, 1948. Thermodynamic analysis of the relative effectiveness of narcotics. *J. Cell. Comp. Physiol.*, **32**: 211–234.

Cole, W. H., and J. B. Allison, 1930. Chemical stimulation by alcohols in the barnacle, the frog, and planaria. *J. Gen. Physiol.*, **14**: 71–86.

Dethier, V. G., and L. E. Chadwick, 1948. Chemoreception in insects. *Physiol. Rev.*, **28**: 220–254.

Dethier, V. G., and M. T. Yost, 1952. Olfactory stimulation of blowflies by homologous alcohols. *J. Gen. Physiol.*, **35**: 823–839.

Ferguson, J., and H. Pirie, 1948. The toxicity of vapours to the grain weevil. *Ann. Appl. Biol.*, **35**: 532–550.

Frings, H., 1946. Gustatory thresholds for sucrose and electrolytes for the cockroach, *Periplanata americana* (Linn). *J. Exp. Zool.*, **102**: 23–50.

von Frisch, K., 1924. Sinnesphysiologie der Wassertiere. Verh. deutsch. Zool. Gesellsch., pp. 21–42.

Hodgson, E. S., 1951. Reaction thresholds of an aquatic beetle, *Laccophilus maculosus* Germ., to salts and alcohols. *Physiol. Zool.*, **24**: 131–140.

Jahn, T. L., and V. J. Wulff, 1950. Chemoreception. In: C. L. Prosser (ed.), Comparative animal physiology. W. B. Saunders, Philadelphia.

Leasure, J. K., 1939. The mucus sheet on the respiratory mucous membrane. *Trans. Amer. Acad. Opthalmol. Otol.*, **44**: 341–347.

Matthes, E., 1924. Das Geruchsvermögen von *Triton* beim Aufenthalt unter Wasser. *Zeitschr. vergl. Physiol.*, **1**: 57–83.

Matthes, E., 1927. Der Einfluss des Mediumwechsels auf das Geruchsvermögen von *Triton*. *Zeitschr. vergl. Physiol.*, **5**: 83–166.

Miller, L. C., and M. L. Tainter, 1944. Estimation of the ED_{50} and its error by means of logarithmic-probit graph paper. *Proc. Soc. Exp. Biol. and Med.*, **57**: 261–264.

Moncrieff, R. W., 1944. The chemical senses. John Wiley & Co., New York.

Murphy, W. E., 1931. Anaphylactic changes in the nasal mucosa. *Arch. Otolaryngol.*, **13**: 842–845.

Nagel, W., 1894. Ergebnisse vergleichend-physiologischer und anatomischer Untersuchungen über den Geruchs- und Geschmäcksinn und ihre Organe. *Biol. Zentrbl.*, **14**: 543–555.

Parker, G. H., and R. E. Sheldon, 1913. The sense of smell in fishes. U. S. Bur. Fisheries Document No. 775.

Richards, A. G., 1952. Studies on arthropod cuticle. VIII. The antennal cuticle of honeybees, with particular reference to the sense plates. *Biol. Bull.*, **103**: 201–225.

Ritter, E., 1936. Untersuchungen über chemischen Sinn beim schwarzen Kolbenwasserkafer *Hydrous piceus*. *Zeitschr. vergl. Physiol.*, **23**: 543–570.

Roth, L. M., and E. R. Willis, 1951. Hygroreceptors in Coleoptera. *J. Exp. Zool.*, **117**: 451–487.

Schaller, A., 1926. Sinnesphysiologische und psychologische Untersuchungen an Wasserkäfern und Fischen. *Zeitschr. vergl. Physiol.*, **4**: 370–464.

Schneirla, T. C., 1952. A consideration of some conceptual trends in comparative psychology. *Psychol. Bull.*, **49**: 559–597.

Snodgrass, R. E., 1935. Principles of insect morphology. McGraw-Hill, New York.

Walker, T. J., and A. D. Hasler, 1949. Detection and discrimination of odors of aquatic plants by the bluntnose minnow (*Hyborhynchus notatus*). *Physiol. Zool.*, **22**: 45–63.